Combining mass spectrometry with evaporative light scattering detection as a powerful tool for analysis of pharmaceuticals

Application Note

Pharmaceuticals

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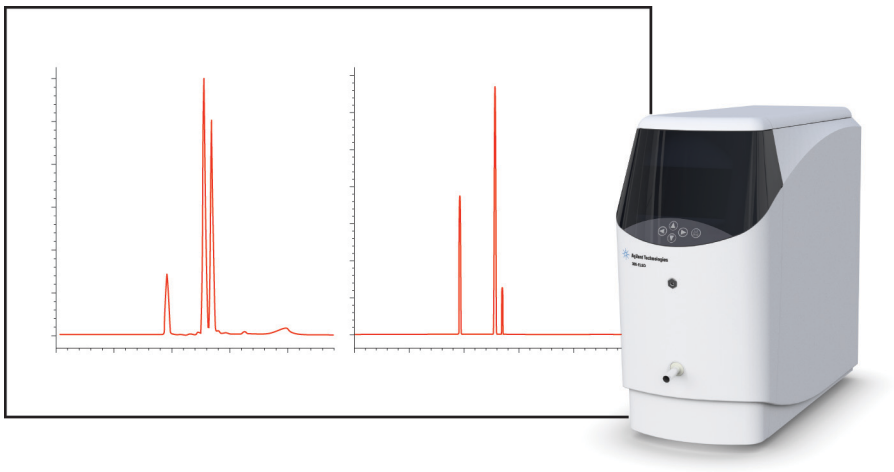

Abstract

This Application Note describes the combination of mass spectrometry and evaporative light scattering detection as a powerful tool for the analysis of pharmaceuticals. An Agilent 500 Ion Trap MS was coupled to an Agilent 385-ELSD Evaporative Light Scattering Detector for quantification of Haloperidol and its metabolites, Diazepam and Verapamil, and Ibuprofen and ß-cyclodextrin. Although mass spectrometry is highly sensitive and widely used as an information rich detector, it is not universal and some analytes do not ionize well using standard ion sources. These problems can be overcome by coupling to another kind of detector such as an evaporative light scattering detector, which has many advantages over refractive index and ultraviolet detectors.

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Introduction

Mass spectrometry (MS) is highly sensitive and widely used as an information rich detector. However, it is not universal. Some analytes do not ionize well using standard MS API sources (ESI and APCI), and ion suppression due to matrix effects is common. These problems can contribute to difficulties in method development.

One way to overcome this is to use simultaneous detection with another kind of detector. Traditionally, refractive index (RI) and ultraviolet (UV) detectors have been widely used in HPLC. Evaporative light scattering (ELS) detection is a better alternative than UV detection at low wavelengths, because its detection is not wavelength-dependent. Also, ELS detection can be used with a wide range of solvents when compared to detection with RI, and is gradient compatible.

The principle of operation of ELS detection employs three distinct stages.

In nebulization, the eluent is mixed with nitrogen or air using a concentric nebulizer design with temperature control. Evaporation of the solvent droplets takes place in the evaporator tube (drift tube) at an independently controlled temperature. Detection is performed by using a high power light emitting diode (LED) and photomultiplier. The ELSD has operating parameters similar to LC/MS, such as the use of volatile mobile phases and flow rates in the range of 0.2–0.5 mL/min.

Three applications are described in this Application Note that demonstrate the usefulness of MS coupled with ELSD:

1. **Haloperidol and its metabolites** — different responses for these compounds from MS and ELS.
2. **Diazepam and Verapamil** —different relative responses between MS and ELS detection.
3. **Ibuprofen and ß-cyclodextrin** — quantification of both compounds without cyclodextrin contamination of the MS ion source.

Experimental

Instrumentation

- Agilent 500 Ion Trap LC/MS equipped with ESI source
- Agilent 385-ELSD Evaporative Light Scattering Detector

The LC/MS-ELS detection system was configured with a low dead volume T-piece for simultaneous detection (Figure 1) or with a divert valve for selected detection (Figure 2).

Materials and reagents

- Haloperidol and metabolites I and II (CAS numbers 52-86-8, 39512-49-7, 34104-67-1)
- Diazepam (CAS number 439-14-5)
- Verapamil (CAS number 152-11-4)
- Ibuprofen (CAS number 51146-56-6)
- α-cyclodextrin and ß-cyclodextrin (CAS numbers 10016-20-3, 68168-23-0) were obtained from Sigma Aldrich, St. Louis, MO.
- All other chemicals were reagent grade or HPLC grade.

Sample preparation

The following mixtures were prepared:

- Haloperidol, metabolite I, metabolite II, 100 µg/mL each in 23:77 methanol:water
- Diazepam and Verapamil, 10 µg/mL each in 50:50 mobile phase A:B
- Ibuprofen and α-cylcodextrin, 10 µg/mL each in 50:50 mobile phase A:B

HPLC conditions

Column:	Pursuit XRs C18, 150 × 2.0 mm, 5 µm
Mobile phase:	A: 0.1% formic acid in water
	B: 0.1% formic acid in acetonitrile
Flow rate:	0.2 mL/min

Injection volume: 10 µL

Mixtures 1, 2 and 3 were injected with the following corresponding LC gradient:

1. 10% B to 85% B in 7 min
2. 20% B to 85% B in 5 min
3. 50% B hold for 1 min, then to 80% B in 7 min

API and MS Parameters

The 500 Ion Trap LC/MS was optimized for each of the compounds by infusion. Scan parameters used in these methods are:

Needle:	5000 V
Spray shield:	600 V
Nebulizing gas:	25 psi
Drying gas:	25 psi at 400 °C
Enhanced scan mode:	5000 Da/s

The Agilent 385-ELSD was also optimized for each compound. Key parameters to optimize are nebulization temperature, evaporation temperature and gas flow rate. ELS detection conditions are shown under all Figures.

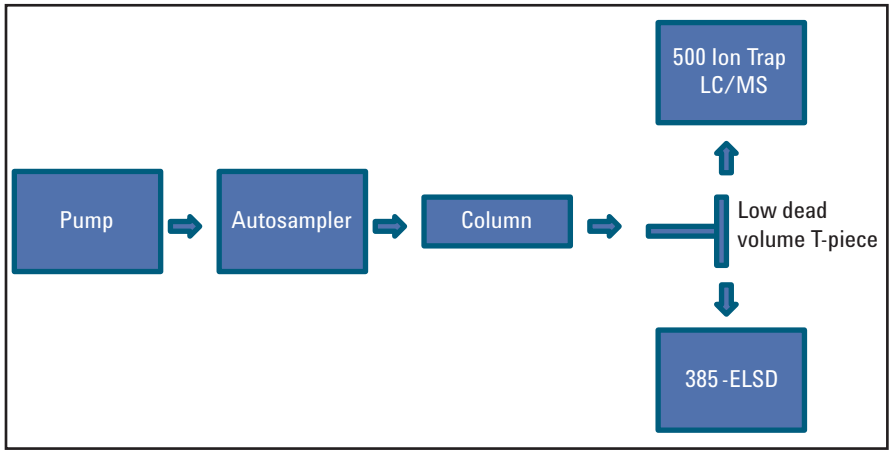


Figure 1
LC/MS-ELS detection system configuration set up for simultaneous detection.

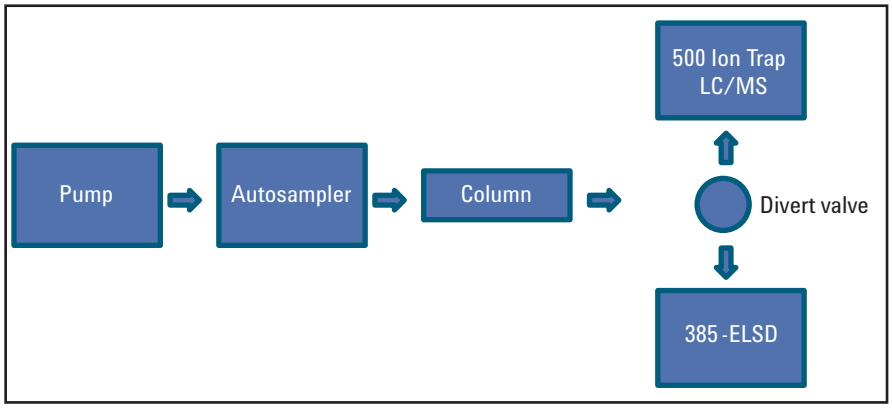


Figure 2
LC/MS-ELSD with divert valve.

Results and discussion

Application (1)

The Agilent 385-ELSD Evaporative Light Scattering Detector shows excellent signal-to-noise and good response for the parent and two metabolites. It can be used in conjunction with the more information-rich MS data. As shown in Figure 3 and Table 1, the detector response can be different than that obtained by MS, enabling optimization of the sensitivity of both instruments.

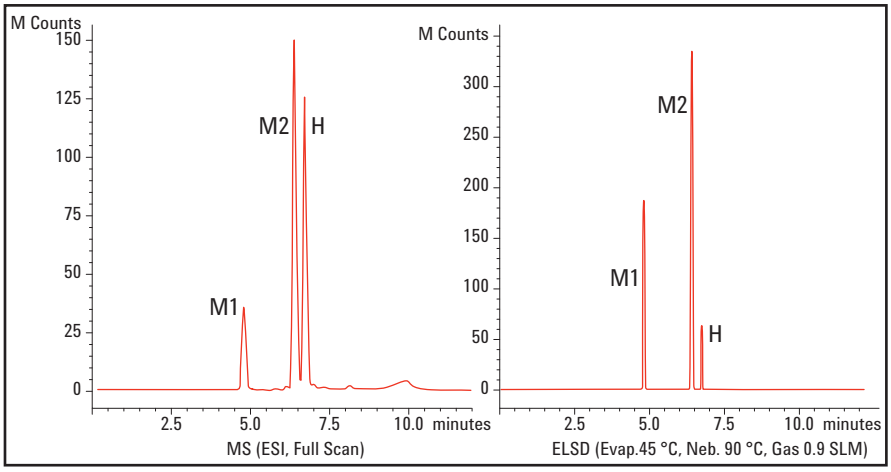


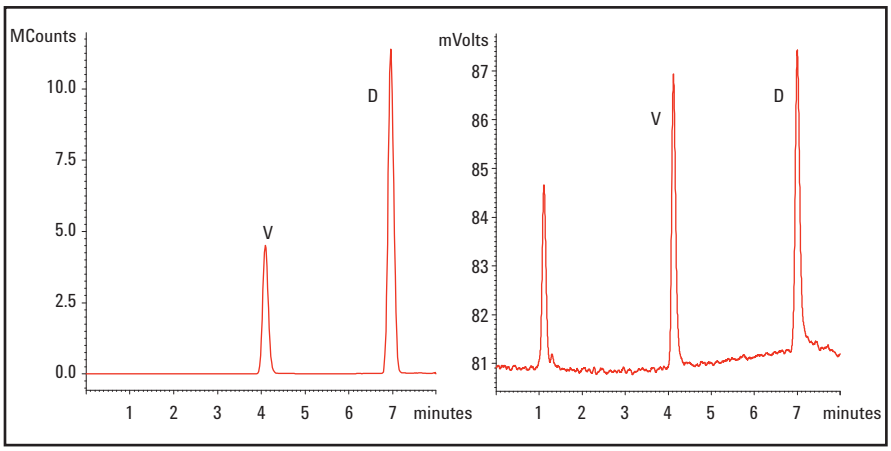
Figure 3
Analysis of a mixture of Haloperidol (H) and metabolites (M1 and M2) using MS and ELS detection.

	MS S/N (full scan)	ELS detection S/N	Sensitivity detection of ELS compared to MS
Metabolite I (peak 1)	1,348	37,400	28×
Metabolite II (peak 2)	2,084	68,000	33×
Haloperidol (peak 3)	1,046	13,000	12×

Table 1
Full scan MS and ELS detection signal-to-noise (S/N) comparison.

Application (2)

Diazepam and Verapamil at the same concentration have significantly different response in full scan MS, but a relatively equal response in the Agilent 385-ELSD Evaporative Light Scattering Detector. Therefore, the 385-ELSD would be useful at the early method development stage. After that, the method can be transferred to MS (Figure 4).



Agilent 500 Ion Trap in MS/MS mode. Response ratio of Verapamil to Diazepam is 1:2

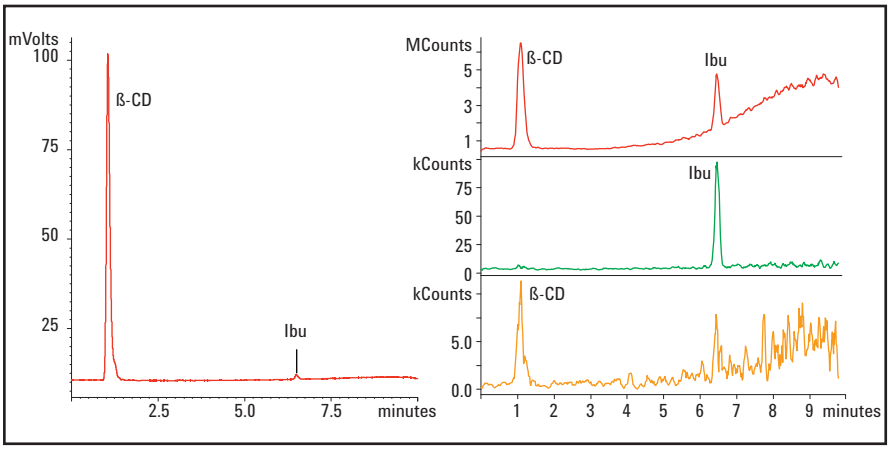
Agilent 385-ELSD Evaporative Light Scattering Detector (Evap. 66 °C, Neb. 90 °C, Gas 1.6 SLM). Response ratio of Verapamil to Diazepam is approximately 1:1.

Figure 4
Mixture of Verapamil (V) and Diazepam (D) (100 ng on each column).

Application (3)

MS detection is more qualitative and quantitative than ELS detection for the active drug (Ibuprofen). However, ß-cyclodextrin, being a sticky excipient, can foul an ESI source fast during an analytical sequence (Figure 5).

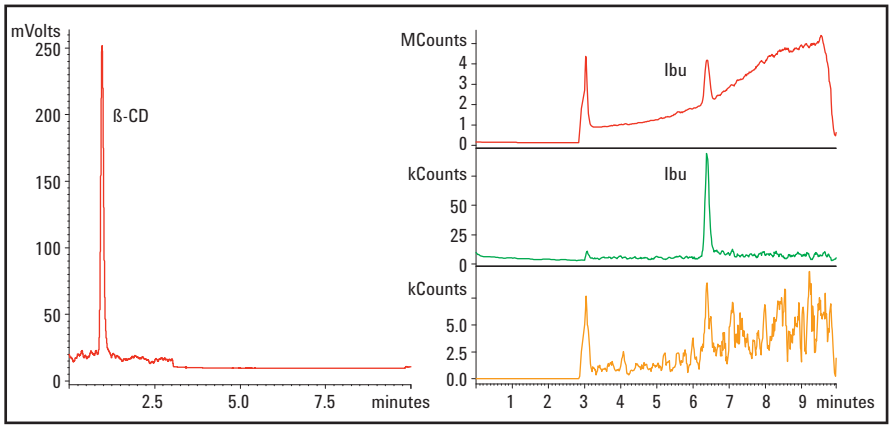
To avoid this problem, the ß-cyclodextrin can be diverted away from the MS source (using the built-in divert valve controlled by the MS workstation software) into the Agilent 385-ELSD Evaporative Light Scattering Detector that is actually more sensitive than MS for this excipient (Figure 2). After the cyclodextrin elutes, the sample is diverted back to the MS for the analysis of Ibuprofen (Figures 6). Table 2 compares sensitivity for the two compounds between the two detectors.



Agilent 385-ELSD Evaporative Light Scattering Detector (Evap. 25 °C, Neb. 90 °C, Gas 1.4 SLM): Sensitive for ß-cyclodextrin, but not Ibuprofen.

Agilent 500 Ion Trap (ESI, Full Scan): XIC is sensitive for Ibuprofen, not ß-cyclodextrin, which also fouls the ion source.

Figure 5
Mixture of Ibuprofen (Ibu) and ß-cyclodextrin (ß-CD) (100 ng each on column) using post-column T (simultaneous detection).



Agilent 385-ELSD Evaporative Light Scattering Detector (Evap.25 °C, Neb. 90 °C, Gas 1.4 SLM): ß-cyclodextrin diverted away from MS.

Agilent 500 Ion Trap (ESI, Full Scan): For Ibuprofen detection only, ß-cyclodextrin diverted to ELSD to keep ion source clean.

Figure 6
Mixture of Ibuprofen (Ibu) and ß-cyclodextrin (ß-CD) (100 ng each on column) using divert valve (selected detection).

	MS (XIC) S/N	ELS detection S/N	More sensitive detector
ß-cyclodextrin	28	930	Agilent 385-ELSD
Ibuprofen	30	15	Agilent 500 Ion Trap LC/MS

Table 2
Sensitivity of LC/MS-ELSD configuration (using post-column T-piece).

Conclusion

This study described the combination of mass spectrometry and evaporative light scattering detection as a powerful tool for the analysis of pharmaceuticals. An Agilent 500 Ion Trap MS was coupled to an Agilent 385-ELSD Evaporative Light Scattering Detector for quantification of Haloperidol and its metabolites, Diazepam and Verapamil, Ibuprofen, and ß-cyclodextrin. The benefits of using ELS detection included:

- Detection of compounds with no chromophore
- Compatible with gradient elution and a wide range of solvents
- No solvent front
- No need for derivatization
- Better alternative to UV at low wavelengths

The advantages of using ELS detection in combination with the Agilent 500 Ion Trap LC/MS included:

- Flow rate range is compatible with ESI or APCI
- Highly complementary with MS for:
  - Simultaneous detection using post-column split
  - Serial/selected detection using the built-in divert valve
  - Early method development to overcome limitations in compound detection in the MS ion source
  - Adding another confirmation technique to the analysis

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